Bacterial Structure and Introduction

Introduction

This first lesson of the Bacteria series throws a lot of information at you fairly quickly. We will engage the content in this lesson in more detail in subsequent lessons, where the relevant information is discussed in the context of the bacterium or the antibiotic. We start off with bacteria in general, then look at Gram-positive versus Gram-negative organisms and discuss the Gram stain; then we look at bacteria from the perspective of their microbiologic features (saving learning them from the perspective of their illness scripts for subsequent lessons). listing microorganism examples as we go. You may feel like you need to memorize this way. We make sure to give you the feature-to-bug perspective first, as a means of introducing and defining terms that will be used later. Then, as we go through the individual bacterial lessons, we give you the bug-to-feature perspective. We teach this first lesson as a means of orientation, but it is NOT how you should start memorizing. It is always better to learn the microbe and its features, rather than memorizing lists of bacteria with a given feature. This is not how other review resources provide the information. This is not what many of your friends did when they studied microbiology. People hate microbiology because there is "so much information." If you have the right perspective (ours), you'll realize it isn't a bunch of memorizing; it isn't endless lists.

This first lesson is what you expect from microbiology and is the orientation lesson. The rest of the microbiology course is what you need.

Inside Bacteria

Bacteria are **prokaryotic**. They do not have organelles. They are single-celled organisms. Their DNA is **chromosomal**, **double-stranded**, and stored in a **nucleoid**—a continuous loop so long that it folds on itself, cluster-like. The nucleoid floats in the cytoplasm, where transcription and translation occur simultaneously. Extrachromosomal DNA also exists in the cytoplasm in the form of **plasmids**. Plasmids are discontinuous from the chromosomal DNA, but are still transcriptionally and translationally active. The cytoplasm is studded with **ribosomes** actively translating RNA into protein.

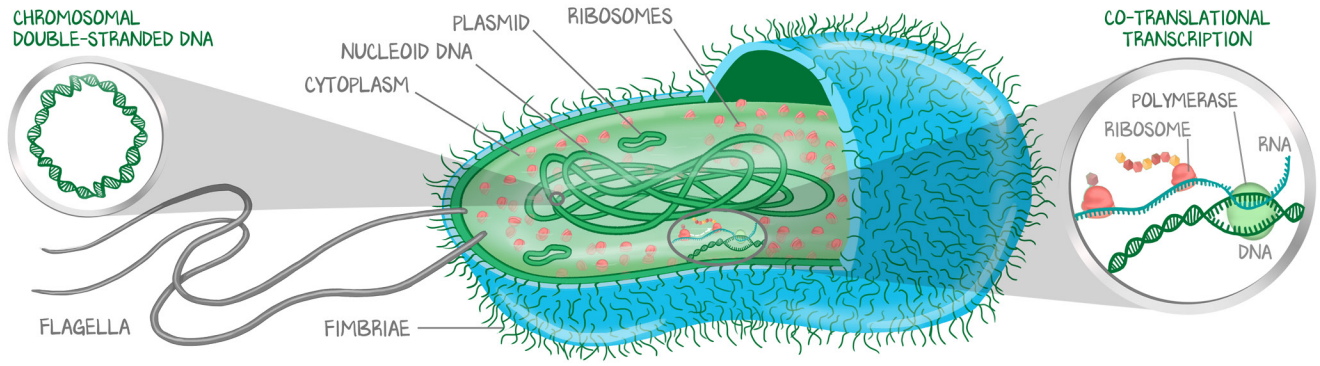

Figure 1.1: Organization of Bacterial Structures
Bacteria are prokaryotic organisms, and so have double-stranded DNA that may undergo cotranscriptional translation. The cytoplasm is filled with ribosomes, proteins, and plasmids. There are no membrane-bound organelles. All bacteria have small appendages called fimbriae or pili. Some bacteria are motile and have flagella.

Outside Bacteria: Gram-Positive vs. Gram-Negative Bacteria

All bacteria have a plasma membrane. Like that of any living cell, this plasma membrane is a lipid bilayer, lipophilic fatty acids in the core hidden from the aqueous environment, hydrophilic heads in contact with the cytoplasm and the extracellular space. The plasma membrane has channels, receptors, and a charge. But very much unlike eukaryotic cells in our bodies, every bacterial cell is a discrete individual. Whereas human cells are organized into layers, and have an extracellular matrix that regulates the surrounding environment and is supported by the cell's host organism, bacteria do not have that help.

Bacteria live—are able to survive the osmotic shifts of an unregulated extracellular world—because they have a bacterial **envelope** that grants them their rigid shape and prevents those osmotic forces from taking their effect. We talk at a high level of what the contents of the envelope are, and what separates Gram-negative organisms from Gram-positive organisms, the details coming in the next several sections. These names refer to their appearance when processed with stains humans invented to see them, but these names also reflect the contents of the bacterial envelope, which in turn reflect their physiology.

Cytoplasmic membrane. All bacteria are cells. Cells are lined by a lipid double bilayer called the plasma membrane. That plasma membrane contains the cytoplasm (where the DNA and ribosomes are), so it is called the cytoplasmic membrane. A bacterium's composition is like that of any other cell—the lipid bilayer (lipophilic with lipophilic) contained by polar head groups (hydrophilic) that are in contact with fluid on either side (cytoplasm inside, periplasmic fluid on the outside).

Cell wall. The cell wall is a layer of peptidoglycans. That means long sugar chains cross-linked by proteins (discussed in detail next). There is a lot of cell wall in Gram-positive organisms. It is, in fact, the only layer of physical support and protection outside the cytoplasmic membrane. Gram-negative organisms also have a peptidoglycan cell well, but their cell wall is smaller and their envelope supported by something extra.

Outer plasma membrane. Only Gram-negative organisms have an outer plasma membrane. It is a complete second membrane, a lipid bilayer, and comes with all the functionality and properties of a lipid bilayer. It separates and selects ions and substrates. It provides osmotic and structural support. The space between the inner plasma membrane and the outer plasma membrane is called the periplasmic space.

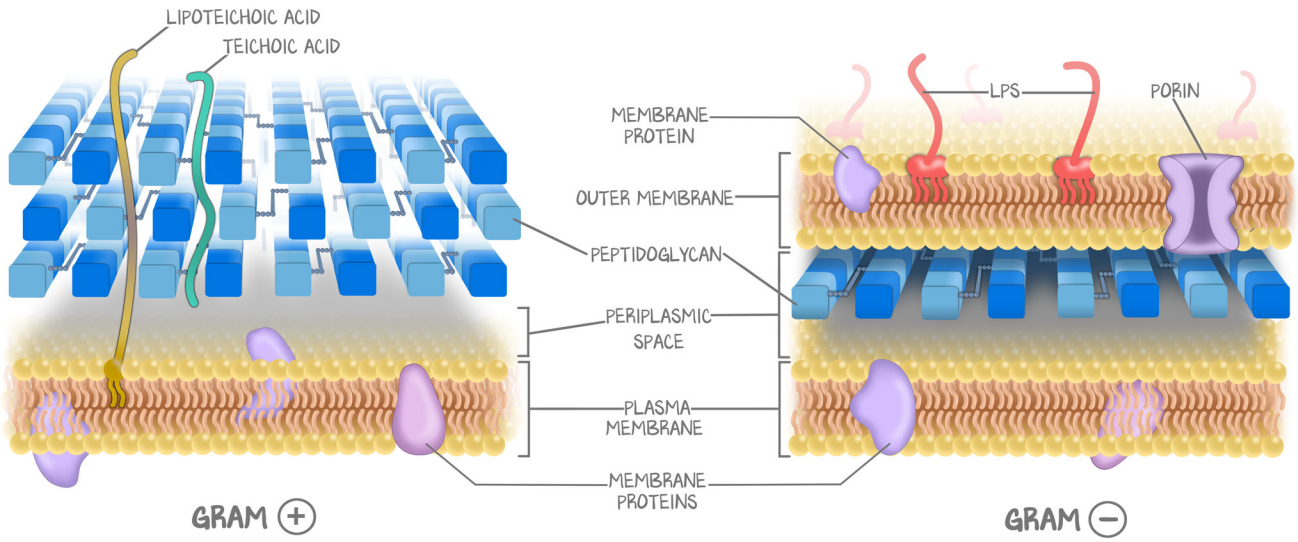

Figure 1.2: Gram Stain Reflects Envelope Structure
The outer layer of protection for Gram negatives and Gram positives is the same size. All of that outer layer of protection in Gram-positive organisms is peptidoglycan cell wall. In Gram-negative organisms, only a small fraction of that outer layer of protection is peptidoglycan cell wall. That is because Gram-negative organisms have the outer membrane instead.

The Cell Wall

All bacteria (except mycoplasma) have a cell wall. The cell wall is made of long chains of **peptidoglycans** (*peptido*, proteins; *glycan*, sugar) cross-linked to each other. On its own, one long string of sugars wouldn't do very much. But when you put a bunch of them next to and on top of each other, all aligned in the same direction, and then connect one chain of sugars to the next, what you get is a scaffolding. The function of the cell wall is to provide **structural support** and to protect against **osmotic pressure**. It is a barrier between the extracellular matrix and the cell membrane. Because there is no outer plasma membrane in Gram-positive organisms, the peptidoglycan cell wall is very thick. Because the Gram negatives have an outer plasma membrane, their cell wall is thin. The cell wall is the site of action of **penicillin-binding proteins** (PBPs). They are named PBPs because they were first studied in their relationship to the mechanisms of action of penicillin. To be clear, PBPs do not bind to penicillin; penicillin binds to and inhibits PBPs. PBPs for a bacterium are the enzymes that synthesize the cell wall, extending the sugar chains and cross-linking the peptides. Penicillin-binding proteins are the bacterial enzymes that **build the cell wall**.

The cell wall of all bacteria is made of peptidoglycan. Peptidoglycan is comprised of polysaccharide chains of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). The NAG-NAM unit is synthesized in the cytoplasm, then attached to other NAG-NAM units outside the cell, forming long chains of sugar. Those long chains of sugar also have spokes of amino acids. The long chains of sugar are cross-linked to neighboring long chains of sugar via the amino acid arms.

Making the sugar chain longer. NAG is synthesized in the cytoplasm. NAM is synthesized in the cytoplasm. A pentapeptide (five-amino-acid-long chain) is added to NAM. At the cytoplasmic membrane, NAM peptide and NAG are attached to each other, and to a carrier called bactoprenol. The entire NAG-NAM peptide is translocated outside the plasma membrane. The NAG-NAM peptide is added onto the already existing peptidoglycan chain using **transglycosylases**. The transglycosylase takes NAG-NAM peptide from the plasma membrane and attaches it to the end of a NAG-NAM chain. This step makes the polysaccharide chain one NAG-NAM peptide longer.

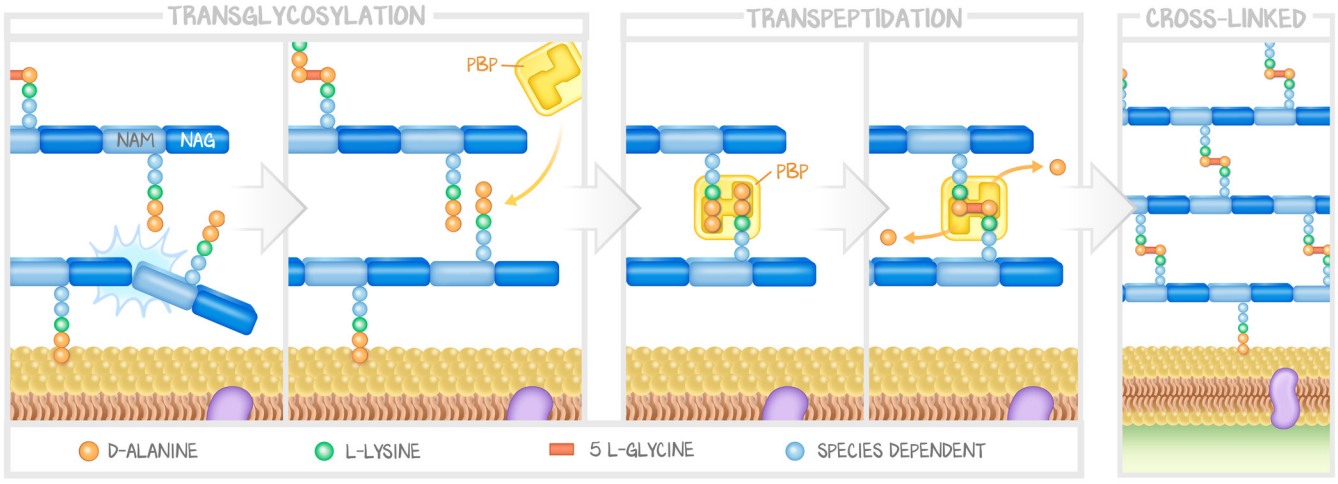

Figure 1.3: Bacterial Cell Wall Synthesis
After bactoprenol translocates the NAG-NAM pentapeptide outside the cell, the NAM is added to the NAG end of the growing glycoprotein peptide via transglycosylation. Penicillin-binding proteins cross-link neighboring sugar chains, utilizing their pentapeptide amino acid sequence. The terminal alanine is removed from one of the pentapeptides, liberating a peptide bond, and the fourth alanine is connected to the third amino acid of the neighboring chain. This process is transpeptidation. The unused terminal alanine of the recipient pentapeptide is cleaved by carboxylation, leaving two connected tetrapeptides.

Cross-linking of neighboring sugar chains. Cross-linking of polysaccharide chains occurs between the pentapeptide arm on NAM of one chain and the pentapeptide arm of NAM of another chain. This cross-linking is called **transpeptidation**, and is carried out by **transpeptidases**. The pentapeptide is cleaved to a tetrapeptide by **carboxypeptidase** and the tetrapeptides are connected to each other by **transpeptidase**. The transpeptidases and carboxypeptidases that are responsible for construction of the peptidoglycan cell wall are collectively referred to as **penicillin-binding proteins** (PBPs; see above for etymology). We will not again distinguish between transpeptidation, carboxypeptidation, or PBPs—in this module, they are synonymous.

The process of cross-linking involves very specific steps with specific amino acids. All bacteria start with a five-amino-acid-long chain attached to NAM. The first two amino acids, the closest to NAM, vary by species. The third is always **lysine**. After lysine is **alanine** and then another **alanine**. The sequence **Lys-D-Ala-D-Ala** is THE sequence to memorize. PBPs recognize D-Ala-D-Ala and cross-link two chains. The details of bond breakage and formation, of the way it happens in bacteria, are not discussed. Certain bacteria do it one way (pentapeptides to each other), and certain bacteria do it another way (chain of five glycines between the pentapeptides).

NAG-NAM forms the sugar backbone chain, connected to other NAG-NAMs by transglycosylation (sugar backbone). The amino acid arms connect the sugar backbone chains to one another, linked chain to chain via amino acids by transpeptidation (peptide).

Gram-Negative Outer Membrane

In **Gram negatives only** there is an **outer plasma membrane**. It is a second lipid bilayer. Lipid bilayers are powerful tools (General Physiology topic). Gram negatives have two of them. The outer membrane is seen only in Gram-negative organisms and not in any other form of life on Earth. Because only Gram negatives have two membranes, only Gram negatives can have a space between two membranes. That space is the **periplasmic space** and is where the cell wall is located. There is an inner leaflet in touch with the periplasmic space and an outer leaflet in touch with the external environment. Spanning the outer membrane are porins. **Porins** are just like our cells' membrane channels—they permit the passage of hydrophilic molecules that cannot diffuse through the outer membrane from the extracellular space to the periplasmic space. They move things like sugars, amino acids, and ions. They are relevant because they also permit certain antimicrobial drugs to be delivered to the periplasmic space. They also can be genetically altered to pump antibiotics OUT of the periplasmic space.

The outer plasma membrane consists of phospholipids on the cell side and of **lipopolysaccharides (LPS)** on the extracellular matrix side (see Figure 1.5). Lipopolysaccharides have three parts—lipid A, the core polysaccharide, and the carbohydrate O antigen. **Lipid A** is the lipophilic portion of LPS and is embedded in the membrane. It is also known as endotoxin and is discussed from that perspective in Bacteria #3: *Toxins*. Lipid A, being lipophilic, is normally buried in the membrane, in the lipophilic portion of the lipid bilayer. It is attached to a **core polysaccharide**, which acts as a bridge between the lipophilic lipid A and the hydrophilic O antigen.

The **O antigen** is a **carbohydrate string** that reaches out from the plasma membrane into the extracellular matrix (think O for Outside). The O antigen is unique to each species, differing by the number and type of sugars (usually 1–50 sugars long). As an example, *E. coli* caused an outbreak of bloody diarrhea when a fast-food chain failed to cook its meat thoroughly. The strain of *E. coli* that did that was named O157:H7. O157 = the 157th known O antigen.

The O antigen is hydrophilic, on the surface of the outer plasma membrane, and exposed to the aqueous environment. It is foreign. The O antigen is antigenic and can induce the formation of antibodies. The formation of antibodies is useful for the detection of organisms (we assess for antibodies to a bacterium

to assess whether the patient has been exposed) but can also cause disease (antigenic mimicry). The O antigen is sugar, which is not as antigenic as a protein. O antigens are what our immune system can respond to when the infection is present. The O antigens are generally poor targets for immunity, so are not used to make vaccines.

Bacteria take two main shapes—long rods, and circles. Their shape is rigid because of their cell wall. **Rod-shaped** bacteria are called bacilli (a bacillus). Bacillus means rod. **Circle-shaped** bacteria are called cocci (a coccus).

Bacteria take two main colors—purple and red/pink. Purple are Gram positives, red/pink are Gram negatives. We now explore the Gram stain and what it means for the bacteria.

Gram Staining

You do NOT have to know the steps for conducting a Gram stain. This paragraph is included so you visualize the process and draw the logical conclusion stated in the next paragraph. Read this paragraph first, using Figure 1.2 to imagine what would happen as the steps are carried out. The first step of Gram staining is to apply crystal violet dye. The crystal violet dye stains purple both the outer plasma membrane of Gram negatives and the cell wall of Gram positives. It would stain the peptidoglycan cell wall of Gram-negative organisms purple also, but cannot penetrate the lipid bilayer (the outer plasma membrane). BOTH Gram-positive organisms and Gram-negative organisms stain purple with crystal violet dye. The second step is to add a lipid solvent (acetone-alcohol). Lipid solvent means "*removes outer lipid layers*." Since Gram positives don't have an outer lipid layer to remove (they have only a peptidoglycan cell wall), nothing is removed from the Gram positives, and so they retain their crystal violet dye. Gram-positives remain purple. Gram-negative organisms have a lipid bilayer that was stained by the crystal dye. Now the lipid solvent removes the outer plasma membrane, removing the thing that was stained. Gram-negatives stain purple with crystal violet but then go colorless again when the lipid solvent is applied. Finally, the third and final step, another dye is added. It is called safranin, also called red counterstain dye. Any peptidoglycan cell wall that is exposed to this red dye will take up the red color. For Gram positives, the cell wall is deeply saturated with the dark purple crystal violet dye. It absorbs the red dye, but the purple color is so much darker than the red dye, that all that is seen is purple. For Gram negatives, the cell wall was never stained by crystal violet, protected by the outer membrane; the outer membrane was stained purple, and the outer membrane was removed. The now exposed peptidoglycan layer takes up the red dye. The only color Gram negatives now have is the red color, which appears pink under light microscopy.

Gram positives stain dark purple because the crystal violet stains their thick cell wall and they have no outer membrane to be dissolved away. **Gram negatives stain light pink** because the crystal violet stains their outer membrane, which is removed by lipid solvent, and their exposed inner cell wall is then counterstained with a red dye.

	GRAM POSITIVE	GRAM NEGATIVE	MYCOBACTERIUM
Crystal violet	Purple	Purple	Colorless
Acetone alcohol	Purple	Colorless	Colorless
Safranin	Purple	Pink	Colorless or light pink

Table 1.1: Gram Stain Steps and Outcomes

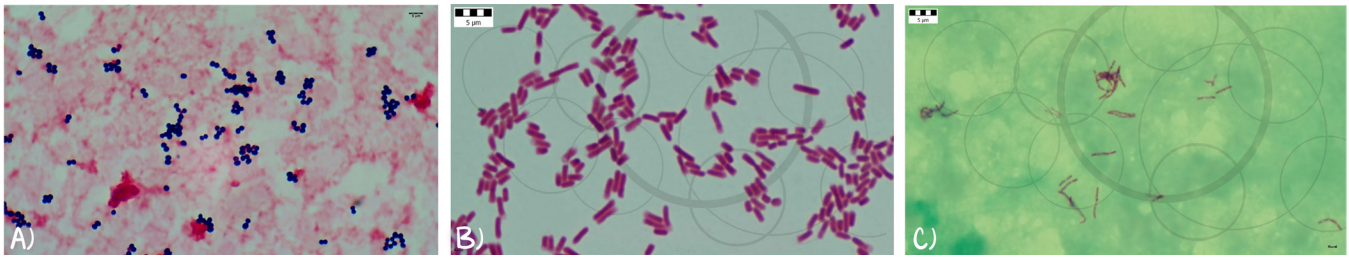

Figure 1.4: Gram Stains
(a) A blood culture showing *Staph. aureus*. The organisms are circular, dark purple (Gram-positive), and are clustered together. They are visible against a pink background (an artifact of the slide preparation). There are several red blood cells on the slide as well. (b) A blood culture of *Escherichia coli*. The organisms are pink (Gram-negative) and elongated (rods). (c) *Mycobacterium tuberculosis* does not stain with the Gram stain. Instead, this acid-fast stain reveals strings of reddish organisms that could be mistaken as Gram-negative rods.

But oh, drat. Science is never that cut and dried. There are some genera that don't behave. *Mycoplasma* does not have any cell wall, so doesn't stain. Some bacteria are too small to be seen. And then there is *Mycobacterium*—every mycobacterial species. Mycobacteria DO have a peptidoglycan cell wall, so it is POSSIBLE that the cell wall gets stained in this process. Mycobacteria do not have an outer plasma membrane. Instead, on top of their peptidoglycan cell wall they have an additional layer of mycolic acid. The lipid solvent does a good job at removing the outer membrane of Gram negatives. It does a poor job of removing mycolic acids from mycobacteria, but it CAN. The crystal violet dye does NOT stain mycolic acid. The safranin red dye does not stain mycolic acid, but can stain exposed peptidoglycans. But since the mycolic acid layer is so thick, the detergent effect of acetone and alcohol is too weak against mycolic acid, and mycobacteria generally do not stain at all. But when they do, they stain **weakly Gram negative**. For mycobacteria with all that mycolic acid, a specialized stain is used. An **acid-fast** stain, a carbol fuchsin stain, is used specifically to stain mycolic acid.

BUG	REASON	ALTERNATE
Mycobacterium genus	Mycolic acid	Acid-fast (carbol fuchsin)
Treponema pallidum	Too thin to see	Darkfield, fluorescent antibody
Mycoplasma pneumonia	No cell wall, very small	None
Legionella	Poor uptake of safranin	Prolonged safranin stain time
Chlamydia	Intracellular, very small, limited muramic acid	Giemsa stain
Rickettsia	Intracellular, very small	Giemsa stain
Ehrlichia	Intracellular, very small	Giemsa stain

Table 1.2: Exceptions to Gram Staining

STAIN	FEATURE	ORGANISMS
Giemsa	Intracellular	*Chlamydia, Rickettsia*
Periodic acid–Schiff	Glycogen	*Tropheryma whipplei*
Acid-fast stain (carbol fuchsin)	Mycolic acid	TB, *Nocardia, Actinomyces*
India ink	N/A	*Cryptococcus*
Silver stain	Fungi	*Pneumocystis jirovecii* (PCP)

Table 1.3: Special Stains
There are even more stains beyond Gram-positive, Gram-negative, and acid-fast. We will discuss specific diagnostic tests, including specialized stains, when we discuss the organism.

External Structure of Bacteria

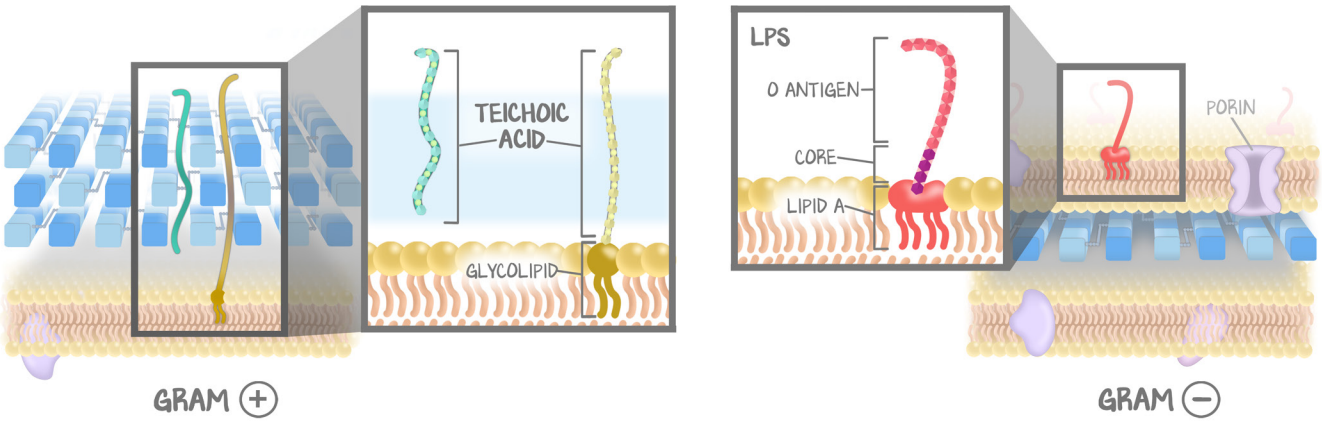

Figure 1.5: Details of the External Bacterial Structures
Gram-positive organisms have a large peptidoglycan cell wall and teichoic acid. Gram-negative organisms are more complex. They also have a peptidoglycan cell wall but also a second outer membrane embedded with lipopolysaccharide and featuring porin channels that restrict access to the periplasmic space.

Pili (sing. pilus) or fimbriae. Pili are made of glycoprotein (sugar and protein), are extracellular structures, and allow the bacterium to reach out into the environment around it. Pili serve two functions. The first function is **attachment to epithelial surfaces**, a necessary first step in establishing infection of host tissue. The second function is as a sex pilus, which mediates the attachment of two bacteria together during conjugation. The sex pilus allows for the cytoplasms of two bacterial cells to become one in order to exchange genetic material (Bacteria #2: *Bacterial Genetics*). Most Gram negatives have pili. The third function of pili is to allow Gram-negative movement.

Flagella. Flagellated organisms have long tails made of microtubules. Flagellated organisms are motile. The flagellum rotates like a fan, generating motile force. Motility is a defining feature for any bacteria that have it. Most bacteria are nonmotile and nonflagellated.

Spores. Spore-forming organisms are intensely difficult to eradicate. Spores are composed of a thick **keratin-like coat** that contains **dipicolinic acid**. When nutrients are limited, the bacteria go dormant. Spores are a hibernation state for bacteria. They provide **resistance to dehydration**, **heat**, and **chemicals**. In the spore form, bacteria are able to survive many years in soil without any supply of nutrients.

They can survive so long because they lack metabolic activity. When re-exposed to nutrients, they can germinate back into bacteria. **Only Gram-positive** organisms form spores (i.e., *most* Gram-negatives do not), particularly species of the *Clostridium* and *Bacillus* genera (which we will discuss in detail in Bacteria #13: *Gram-Positive Rods*).

Plasmids. All bacteria have plasmids. Plasmids are **extrachromosomal DNA** that exist in the cytoplasm and are circular and double-stranded. They are distinct and independent from the chromosomal DNA of the nucleoid, also in the cytoplasm. Transcription and replication of plasmids happen in the cytoplasm, also independent of the chromosomal DNA. Plasmids are the means of developing and transmitting **antibiotics resistance** and **toxin formation** (discussed in Bacteria #2: *Bacterial Genetics*).

Capsules. Capsules are on the outermost layer of a bacterial envelope, existing either on top of the peptidoglycan cell wall (Gram positives) or on top of the outer membrane (Gram negatives). Capsules **evade phagocytosis** by preventing phagocyte adherence to the bacteria. Encapsulated organisms can be recalled using the mnemonic: "Some Killers Have Pretty Nice CapsulESS"—*Strep.* (*pneumoniae*, GAS, GBS), *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Neisseria meningitidis*, *Cryptococcus* (fungus), *E. coli* (some strains that cause meningitis), *Salmonella,* and *Staph. aureus*. Encapsulated organisms are normally opsonized and cleared by the **spleen** because they are able to evade phagocytes. If a patient becomes **asplenic** (sickle cell disease, surgical resection following trauma) for any reason, these organisms have an increased virulence. The good news is that these capsules are polysaccharides, foreign antigens we can use to develop **vaccines**. We currently have vaccines against *Strep. pneumoniae* ("pneumonia shot"), *H. flu* ("epiglottitis shot"), and *N. meningitidis* ("meningitis shot"). The capsule polysaccharides are antigenic, but we can make them even more antigenic by conjugating them to a carrier protein. **PCV13** (pneumococcal conjugate vaccine) provokes an IgM response with subsequent class-switching to IgG, whereas the **PPSV23** (pneumococcal polysaccharide vaccine without conjugation) elicits only an IgM response without class-switching to IgG. The *H. flu* and *N. meningitidis* vaccines only come conjugated to a carrier protein. Capsules are made of polysaccharide (sugar) in every bacterium **except for *Bacillus anthracis*** (which is also not in the mnemonic), in which the capsule is a **polypeptide of D-glutamic acid** (protein).

Glycocalyx. The glycocalyx is a component of **biofilms**. The glycocalyx itself is a polysaccharide coat. Biofilms mediate adherence to the surface of human cells. Most importantly, biofilms also allow for increased **adherence to prosthetic equipment**. Organisms with biofilms more easily infect prosthetic joints, prosthetic heart valves, and central venous catheters. Biofilms are very hard to treat with antibiotics, often requiring the infected hardware to be removed.

Virulence

Virulence is a measure of a microbe's ability to **cause disease**. Being present is not the same as causing disease. Which means that once a bacterium reaches a critical threshold, a certain number of organisms, it causes disease. Virulence is a statistically measured feature of the species, corresponding to the **ID_{50}**, the infectious dose, the number of organisms at which 50% of the population will suffer an infectious disease. The more virulent a microbe is, the fewer organisms are required to cause disease. A **lower ID_{50}** means a more virulent microbe. Virulence is determined by multiple factors; the high-yield ones we discuss next.

Adherence to cell surfaces. These features are going to allow the bacteria to adhere to the surface of human cells and, in the case of biofilms, also to inert materials. **Pili** are the main mechanism by which Gram-negative microbes adhere. **Teichoic acid** is the primary mechanism of Gram-positive microbes. **Biofilms** are secreted by *Strep. epidermidis* (catheters), Strep. viridans (endocarditis, dental plaque), and *Pseudomonas aeruginosa* (ventilator-associated pneumonia), among others.

Invasion. The extracellular matrix is made of collagen and hyaluronic acid. **Collagenase** degrades collagen. **Hyaluronidase** degrades hyaluronic acid. This allows organisms possessing these enzymes to move within the subcutaneous space easily and quickly, spreading in the plane of infection. This is most classically illustrated by *Strep. pyogenes* causing necrotizing fasciitis or erysipelas (discussed in detail in Musculoskeletal: Dermatology #14: *Skin and Soft Tissue Infections*). Separately, the enzyme **coagulase** accelerates the formation of a fibrin clot, which walls off the infected area by coating the bacterial colony with a layer of fibrin. This promotes abscess formation. This is most classically illustrated by *Staph. aureus*, colloquially "coag-positive staph," as it is the only species of *Staphylococcus* that has the enzyme coagulase.

Evasion. A major form of immune system evasion is the escape from phagocytes conferred by **capsules** (above). Other antiphagocytic factors are bacterial cell wall proteins and bacterial-secreted IgA proteases. **Protein A** of *Staph. aureus* binds to the Fc portion of IgG and prevents opsonization and complement activation. The **M protein** of *S. pyogenes* is a cell wall protein that shares similar epitopes with human cellular proteins. This permits the bacteria to go undetected, displaying proteins (antigens) that the immune system fails to recognize as foreign. It also underlies the autoimmunity of acute rheumatic fever—if antibodies are made against M protein, and M protein looks like normal human cells' proteins, the antibody made against strep may in fact work against self. Some organisms secrete **IgA proteases** that destroy mucosal IgA, preventing opsonization and complement activation.

Intracellular survival. Living within another cell is conferred by being facultatively intracellular (evasion of immune system) and by the enzyme catalase (an enzyme that fights back against phagocytes after phagocytosis has occurred). A bacterium inside the cytoplasm of another cell will go unnoticed by the immune system. Cells communicate by touching plasma membranes and the associated proteins. Phagocytes cannot peer into the cytoplasm of another. In all cases of **facultative intracellular** organisms, the bacterium induces endocytosis (or even phagocytosis) but **prevents lysosome fusion** with the endosome or **escapes into the cytoplasm**. If the lysosome fuses, some bacteria are **catalase-positive**, which degrades the hydrogen peroxide generated by the respiratory burst of lysosomal killing (Immunology #4: *Innate Immune System*). Catalase degrades the H_2O_2 of the of the oxidative burst before it can be converted by myeloperoxidase into HOCl (bleach). Some bacteria are **obligate intracellular pathogens** and use the host cell to deliver energy and molecular resources. One such bacterium is *Chlamydia*, originally suspected to be a virus, as it is so small and demands so much from its host.

FACULTATIVE INTRACELLULAR	OBLIGATE INTRACELLULAR	CATALASE POSITIVE
Listeria	*Chlamydia* species	***Staphylococcus***
Salmonella	*Rickettsia* species	All *Enterobacteriaceae*
Shigella	*Anaplasma*	*Nocardia*
Neisseria	*Ehrlichia*	*Pseudomonas*
Mycobacterium	*Coxiella*	*Listeria*
		Aspergillus (fungus)
		Candida (fungus)

Table 1.4: Intracellular Survival
A list of organisms and their features. *Enterobacteriaceae = E. coli, Enterobacter, Klebsiella, Shigella, Yersinia, Proteus, Salmonella*, and *Serratia.*

Extracellular survival. One host defense mechanism is acidification of the environment, especially in the stomach and somewhat in the urine. Urea-splitting organisms raise the pH of their environment. Urea-splitting organisms possess the enzyme **urease** (urea-ase). Classic examples of urease-producing organisms are ***Klebsiella*** and ***Proteus***, which cause urinary tract infections and struvite kidney stones, and ***H. pylori***, causing both acute and chronic gastritis as well as peptic ulcers. Some bacteria use complex carbohydrate metabolism, akin to a rudimentary version of our mitochondrial electron transport chain. Those organisms that use oxygen as an electron acceptor use the enzyme **oxidase**. Others are able to preferentially **ferment sugars** to generate energy. As we will see in Bacteria #4: *Laboratory Diagnosis*, we can assess the presence of urease and oxidase to facilitate diagnosis. Extracellular survival is also conferred by spores (above), and, as will be discussed (in Bacteria #3: *Toxins*), exotoxins.